



PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Perryman, Benjamin M., *et al.*

Serial No.: 10/697,991

Filed: 10/30/2003

For: QUANTITATIVE ANALYSIS OF
PROTEIN ISOFORMS USING MATRIX-
ASSISTED LASER
DESORPTION/IONIZATION TIME OF
FLIGHT MASS SPECTROMETRY

Group Art Unit: 1743

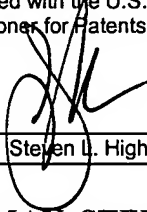
Examiner Gakh, Yelena G.

Atty. Dkt. No.: MYOG:056US

CERTIFICATE OF MAILING
37 C.F.R. § 1.8

I hereby certify that this correspondence is being deposited with the U.S. Postal Service with sufficient postage as First Class Mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, Va 22313-01450, on the date below:

January 19, 2005
Date


Steven L. Highlander

DECLARATION OF M. BENJAMIN PERRYMAN, STEVE M. HELMKE AND MARK W.

DUNCAN UNDER 37 C.F.R. §1.131

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-01450

Dear Sir:

We, M. Benjamin Perryman, Steve M. Helmke, and Mark W. Duncan, do declare the following:

1. M. Benjamin Perryman is a citizen of the United States. M. Benjamin Perryman resides at 25638 487th Avenue, Garretson, SD 57030, and currently holds the position of Director at South Dakota Health Research Foundation, Sioux Falls, SD. Steve M. Helmke is a citizen of the United States. Steve Helmke resides at 2737 Hooker Street, Denver, CO 80211, and currently holds the position of Instructor at the University of

Colorado, Denver, CO. Mark W. Duncan is a citizen of Australia. Mark W. Duncan resides at 4550 Lowell Blvd., Denver, CO 80211, and currently holds the position of Professor at the University of Colorado.

2. We are the named inventors in the above-captioned application, U.S. Serial No. 10/697,991, entitled "QUANTITATIVE ANALYSIS OF PROTEIN ISOFORMS USING MATRIX-ASSISTED LASER DESORPTION/IONIZATION TIME OF FLIGHT MASS SPECTROMETRY."
3. We began work using MALDI-TOF for protein isoform analysis prior to Jan. 15, 2002, as evidenced by the papers attached.
4. I hereby declare that all statements made of my own knowledge are true and all statements made on information are believed to be true and further that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issued thereon.

Jan. 18, 2005

Date

M. Benjamin Perryman

M. Benjamin Perryman

Date

Steve M. Helmke

Colorado, Denver, CO. Mark W. Duncan is a citizen of Australia. Mark W. Duncan resides at 4550 Lowell Blvd., Denver, CO 80211, and currently holds the position of Professor at the University of Colorado.

2. We are the named inventors in the above-captioned application, U.S. Serial No. 10/697,991, entitled "QUANTITATIVE ANALYSIS OF PROTEIN ISOFORMS USING MATRIX-ASSISTED LASER DESORPTION/IONIZATION TIME OF FLIGHT MASS SPECTROMETRY."
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Date

1-18-05

Date

M. Benjamin Perryman

Steve M. Helmke

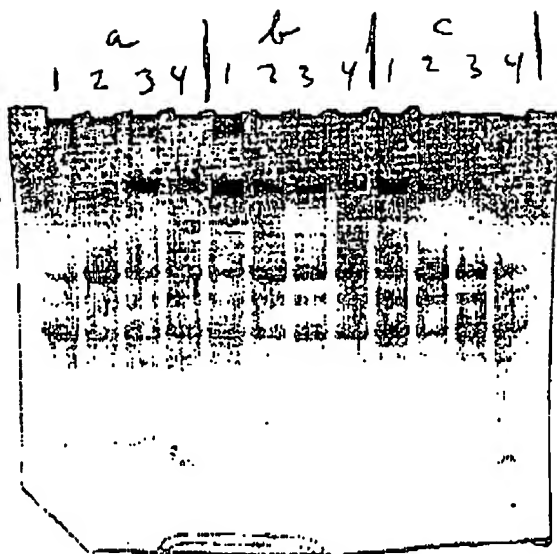
Steve M. Helmke

1/18/51
Date

Mark W. Duncan
Mark W. Duncan



myosin



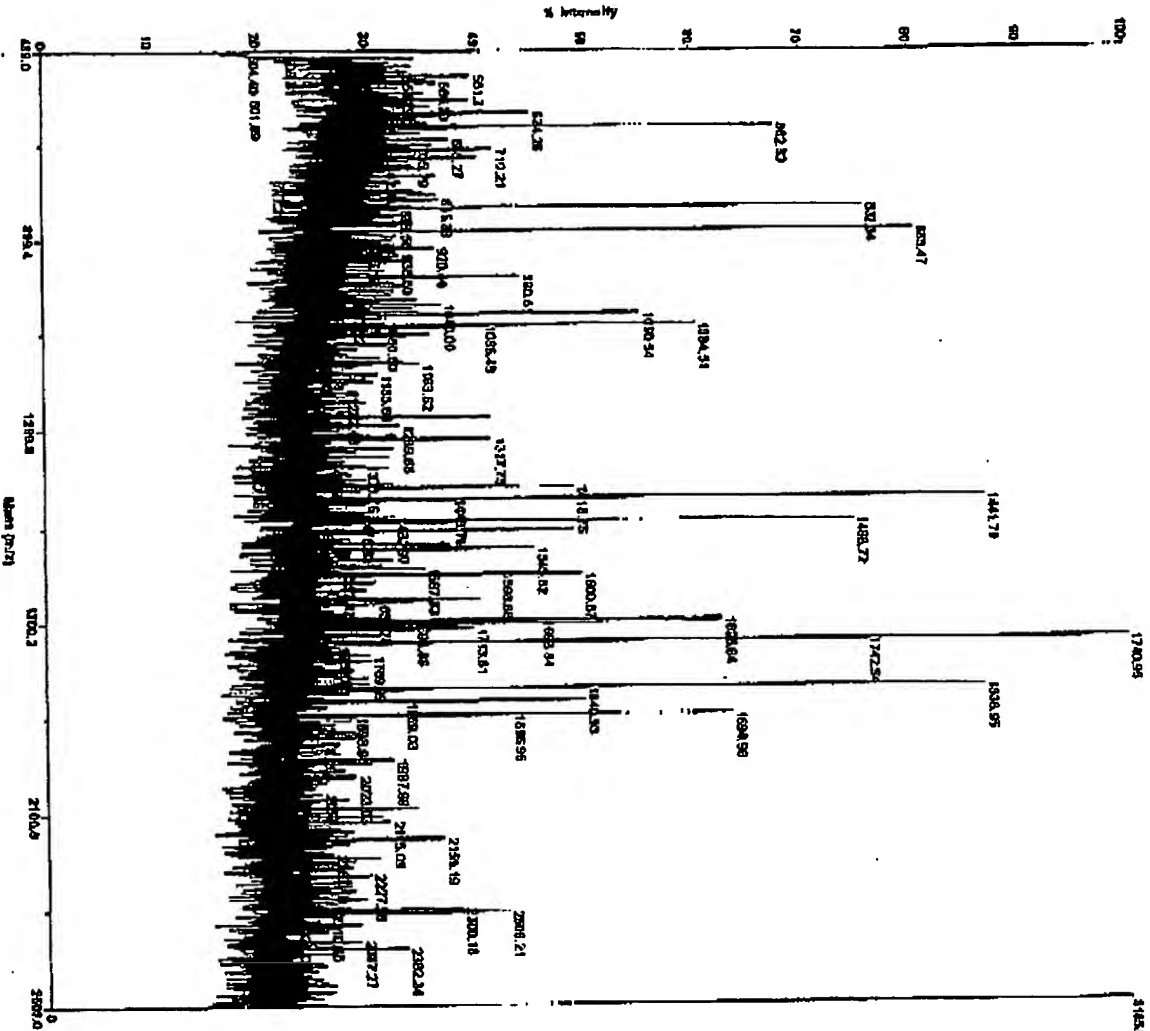
<u>% α-M₂HC (By Az stain)</u>		
1	SMC 18	13
2	Spencer	25
3	GJ1	56
4	Arner	71

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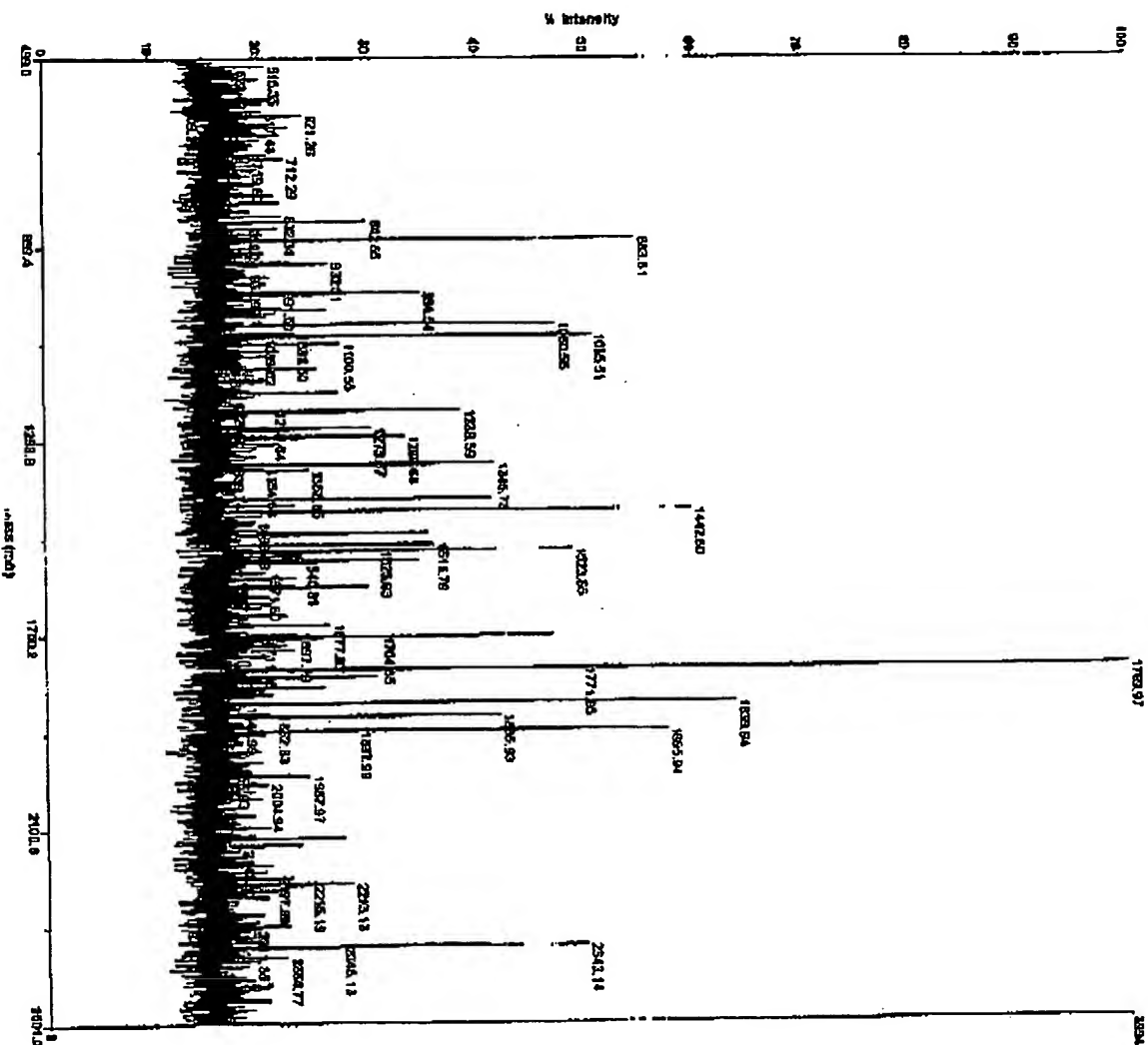
Applied Biosystems Voyager System 6137

Voyager Spec #115P = 1740 B, 31481



Mode of operation:	Reflector
Extraction mode:	Delayed
Polarity:	Positive
Acquisition control:	Manual
11500 Accelerating voltage:	20000 V
Grid voltage:	70%
Mirror voltage ratio:	1.12
Grids wire O:	0%
Extraction delay time:	100 msec
Acquisition mass range:	500 - 2500 Da
Number of laser shots:	100/spectrum
Laser intensity:	2275
Laser Rep Rate:	5.0 Hz
Collision type:	External - WYVAGEPROFRO Diethylamine 11201 ref. col
Collision matrix:	a-Cyano-4-hydroxybenzoic acid
Low mass gate:	500 Da
Timed ion selector:	Off
Digitizer start time:	22.5465
8th rate:	0.5 msec
Number of data points:	55422
Vertical scale O:	1000 mV
Vertical offset:	0%
Input bandwidth O:	750 MHz
Sample well:	45
Plate ID:	PLATE1
Serial number:	8137
Instrument name:	Voyager-DE PRO
Plate type filename:	C:\VOYAGER\100 well plate.pdf
Lab name:	Biochemical Mass Spectrometry Facility
Absolute x-position:	21613.7
Absolute y-position:	25583.3
Relative x-position:	-293.82
Relative y-position:	-404.181
Shots in spectrum:	100
Source pressure:	1.476e-007
Mirror pressure:	3.411e-015
TC2 pressure:	0.011
TIS gate width:	8
TIS flight length:	873

Voyager Spectral IDP = 1770.0, 3356]



Refractor Debyond Positive Material

20000 V
789475%
112

0%

500 - 2500 Da
100/spectrum

2275

External - WW

2-Cyano-4-tyd

10

314

20,545
05,996

U.S. RESC
56472

1000 MAY

076

750 MHz

五

PLATE I

6137
WATER-TITE R

**VOYAGER-DE
C-MOYAGE**

Biochemical Analysis

1

16828.7
95075

262887.2
1 18584

1.10004
0.00833849

100

1.445e-0807

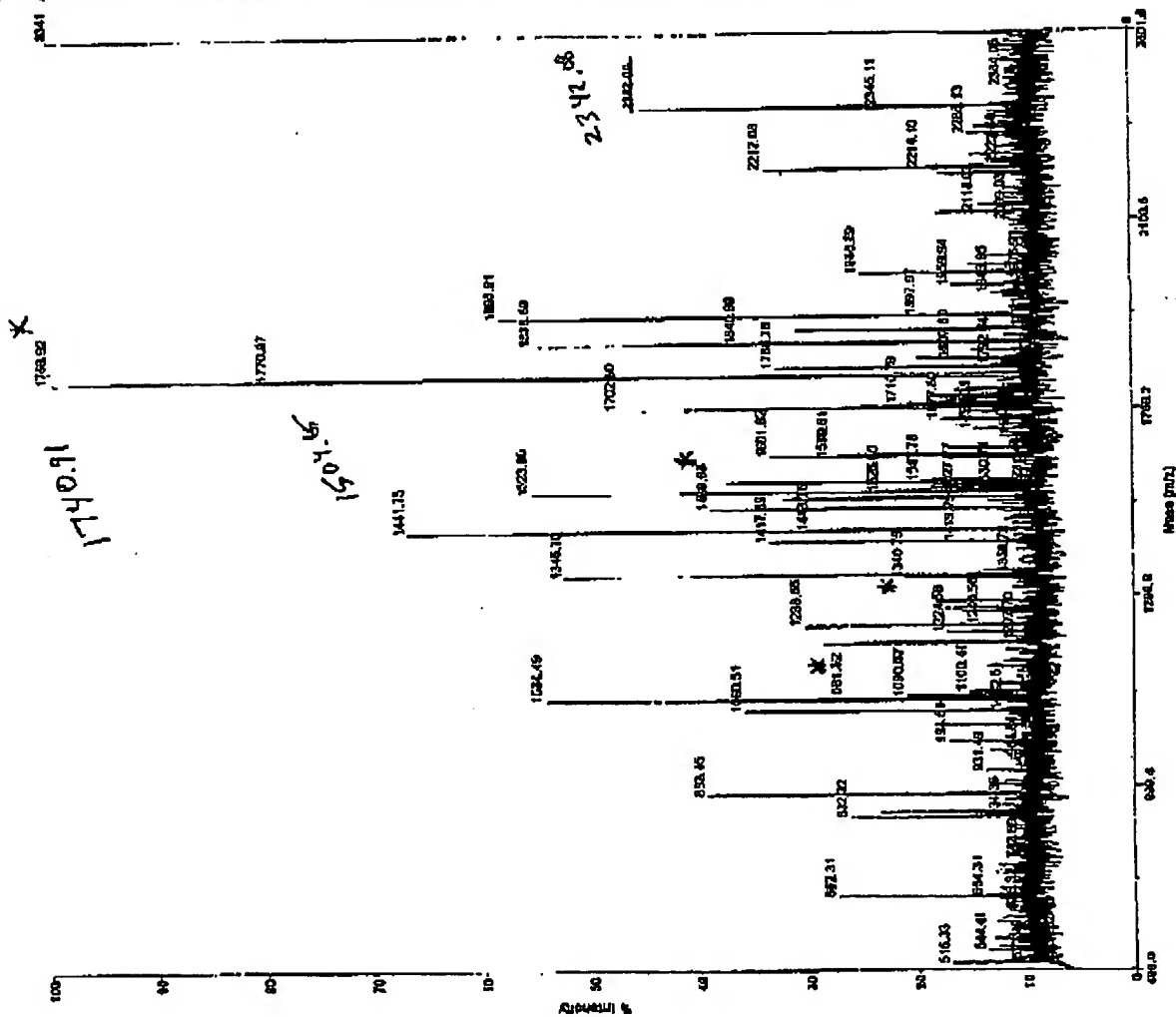
3428a-008
001105

8
09/11/20

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Applied Biosystems Voyager System 6137

Voyager Spec #10-LICBP = 1788.9, 8341)



Modes of operation:
 Extraordinary modes:
 Polarity:
 Acquisition control:
 Reflect
 Delayed
 Possible
 Manual

Accelerating voltage:	20000 V
Grid voltage:	70%
Mirror voltage ratio:	1.12
Guide wire α :	0%
Extraction delay time:	100 nsec

Acquisition mass range: 500 – 2500 Da
Number of laser shots: 1000/spectrum
Laser intensity: 2144
Laser Rep Rate: 5.8 Hz
Calibration type: External – W/O
Calibration method: α -Cyano-4-hydroxymethylbenzoic acid
Low mass gate: 500 Da
Timed ion selector: Off

Digitizer start time:	22.542
Bin size:	0.5 msec
Number of data points:	55420
Vertical scale 0:	1000 mV
Vertical offset:	0%
Input bandwidth 0:	750 MHz

Sample wait
Plate ID:
Serial number:
Instrument name:
Plate type filename:
Lab name:

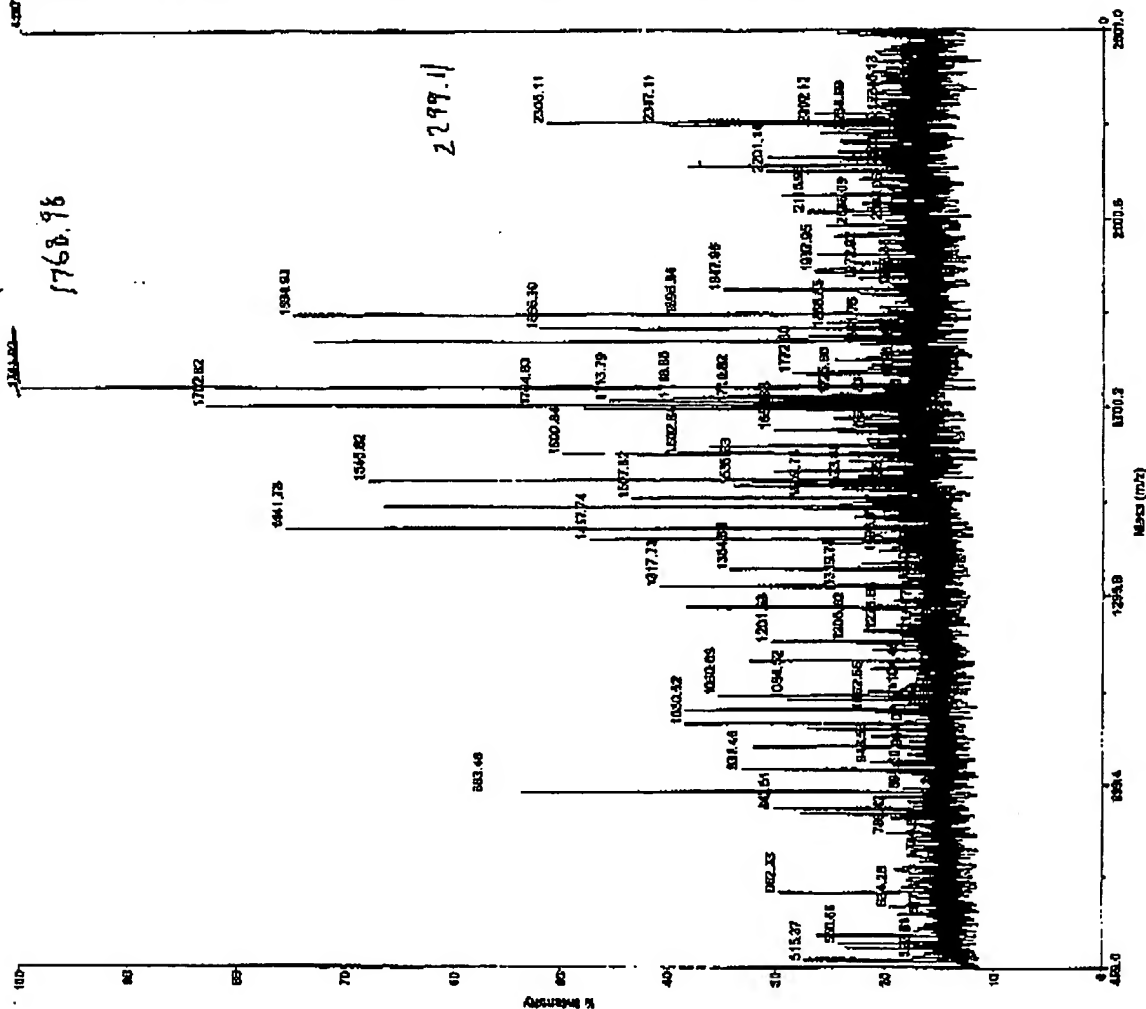
Abcable x-position	17473.5
Absolute y-position	15981.3
Relative x-position	648.011
Relative y-position	-148.155
Shots in spectrum	100
Source pressure	1.798e-0
Mirror pressure	4.514e-0
TC2 pressure	0.05102
TIS gas width	8
TIS flight length	873

64 PLATE1
6137
Voyager-DE PRO
C:\VOYAGER\1100 well plate.pit
Biochemical Mass Spectrometry Facility

1747315
168813
646.011
-148.156
100
1.786e-007
4.514e-008
0.01182
8
873

Applied B' systems Voyager System 6137

Voyager Spec #1 => HC BP = 17419.42887



Mode of operation:	Reflector.
Extraction mode:	Delayed
Polarity:	Positive
Acceleration control:	Manual

Accelerating voltage:	20000 V
Grid voltage:	76%
Mirror voltage ratio:	1.12
Guide wire O:	0%
Extraction delay time:	100 nsec

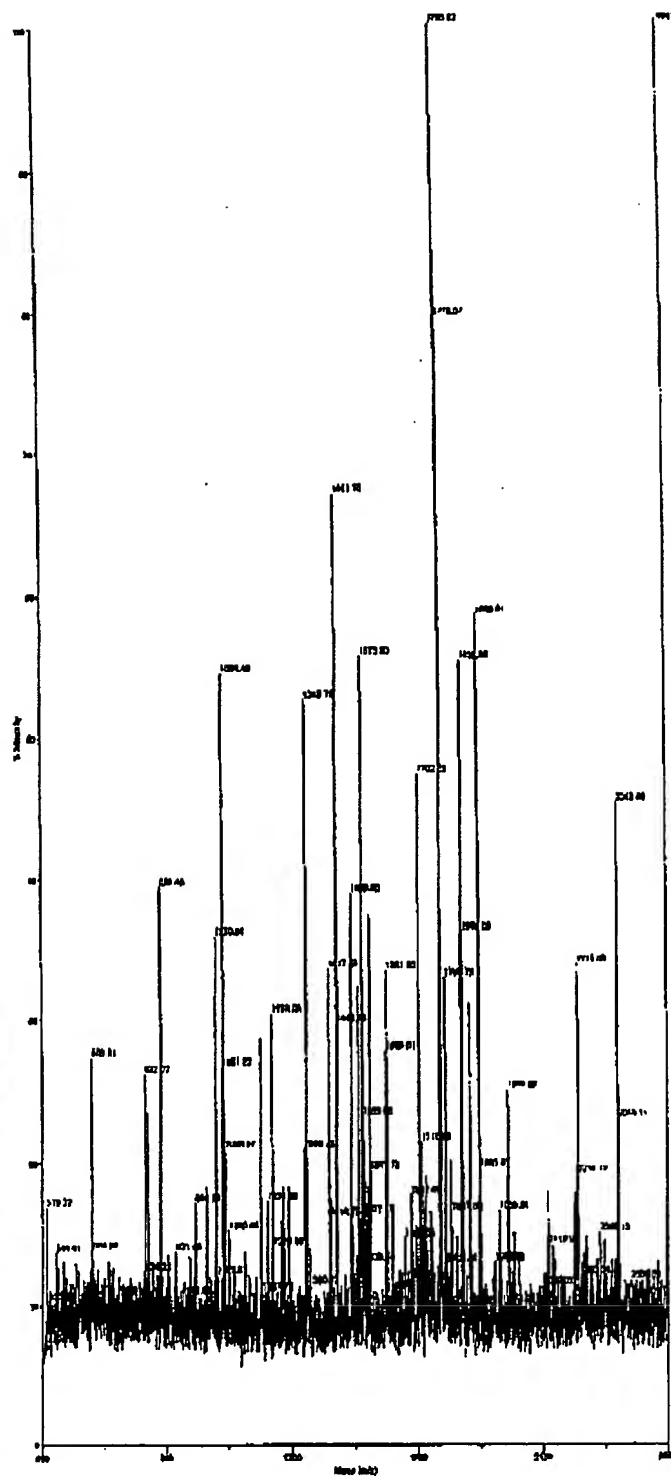
Acquisition mass range:	500 – 2500 Da
Number of laser shots:	100/spectrum
Laser intensity:	1944
Laser Rep Rate:	5.8 Hz
Calibration type:	External – 11Voyager
Calibration matrix:	o-Cyanine-4/tyrosine
Low mass gate:	500 Da
Timed ion selector:	Off

Digitizer start time: 22.542
 Bin size: 0.5 msec
 Number of data points: 55420
 Vertical scale: 1000 mV
 Vertical offset: 0%
 Input bandwidth: 750 MHz

Sample well: 55
Plate ID: PLATE1
Serial number: 8137
Instrument name: Voyager-DE PRO
Pit type filename: C:\VOYAGER\100 well plate.pat
Lab name: Biochemical Nasa Spectrometry Facility

Absolute x-position:	21840
Absolute y-position:	21783.7
Relative x-position:	-387.477
Relative y-position:	-143.759
Shift in spectrum:	100
Source pressure:	1.323e-007
Mirror pressure:	3.816e-008
TC2 pressure:	0.01091
TIS gate width:	8
TIS light length:	673

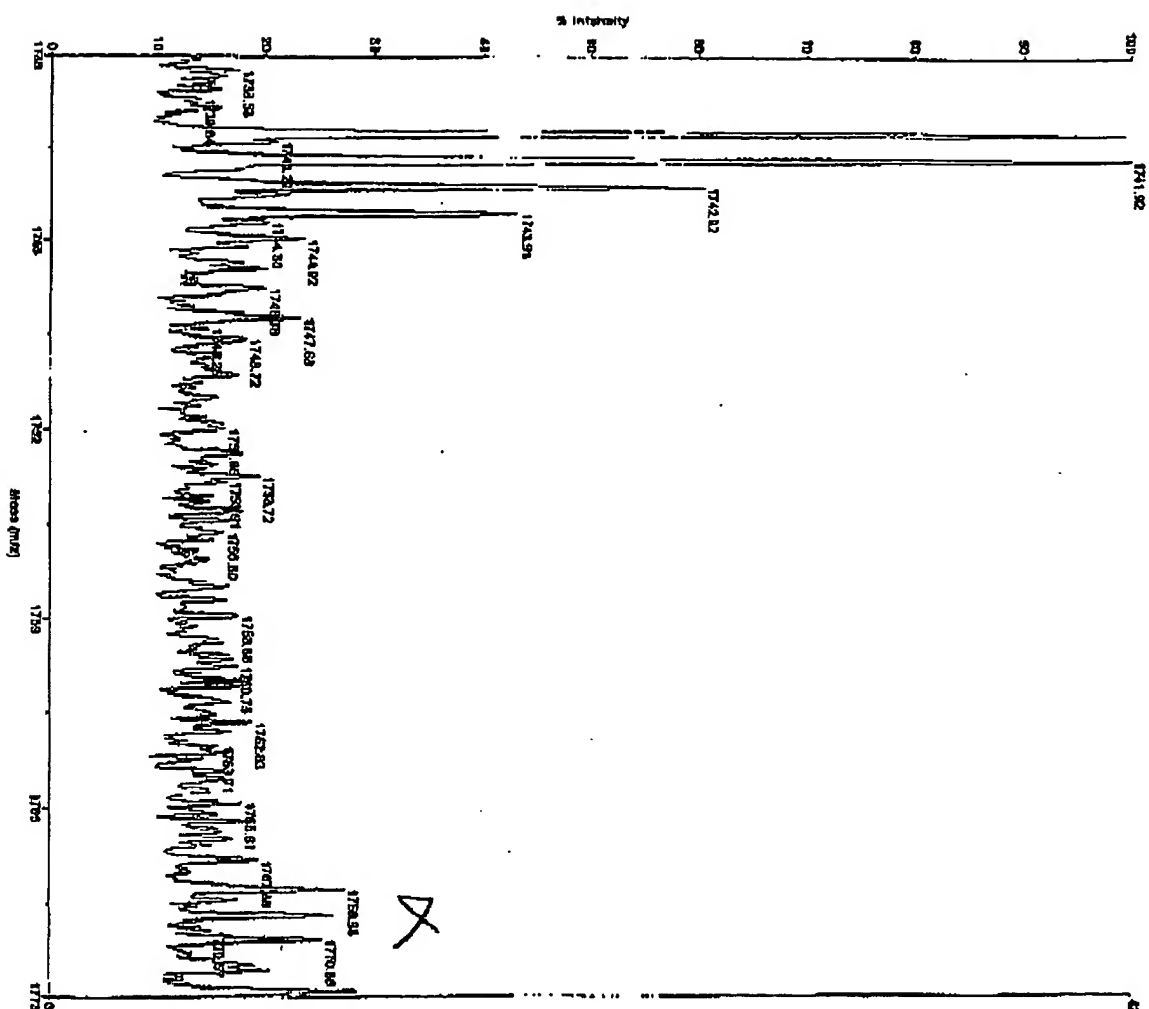
Wappler Rpt 61 000000 = 1722.8, 634 92



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13
1740.94

13
1740.94



Reflector Delayed Positive Manual

200004
7884

1.12 cm

100 msec

500 - 2500 D-

1949

External - W:\orgapro\PRO_Data\Intake\11301_0001.cad

500 Da

1

0.5 msec

1000 ml

750 MHz

PLATE 1

Voyager-

Biochemical Mass Spectrometry Facility

21540

2100.1
367.47

100

3.816e-0

Q. 1081

673

2

2

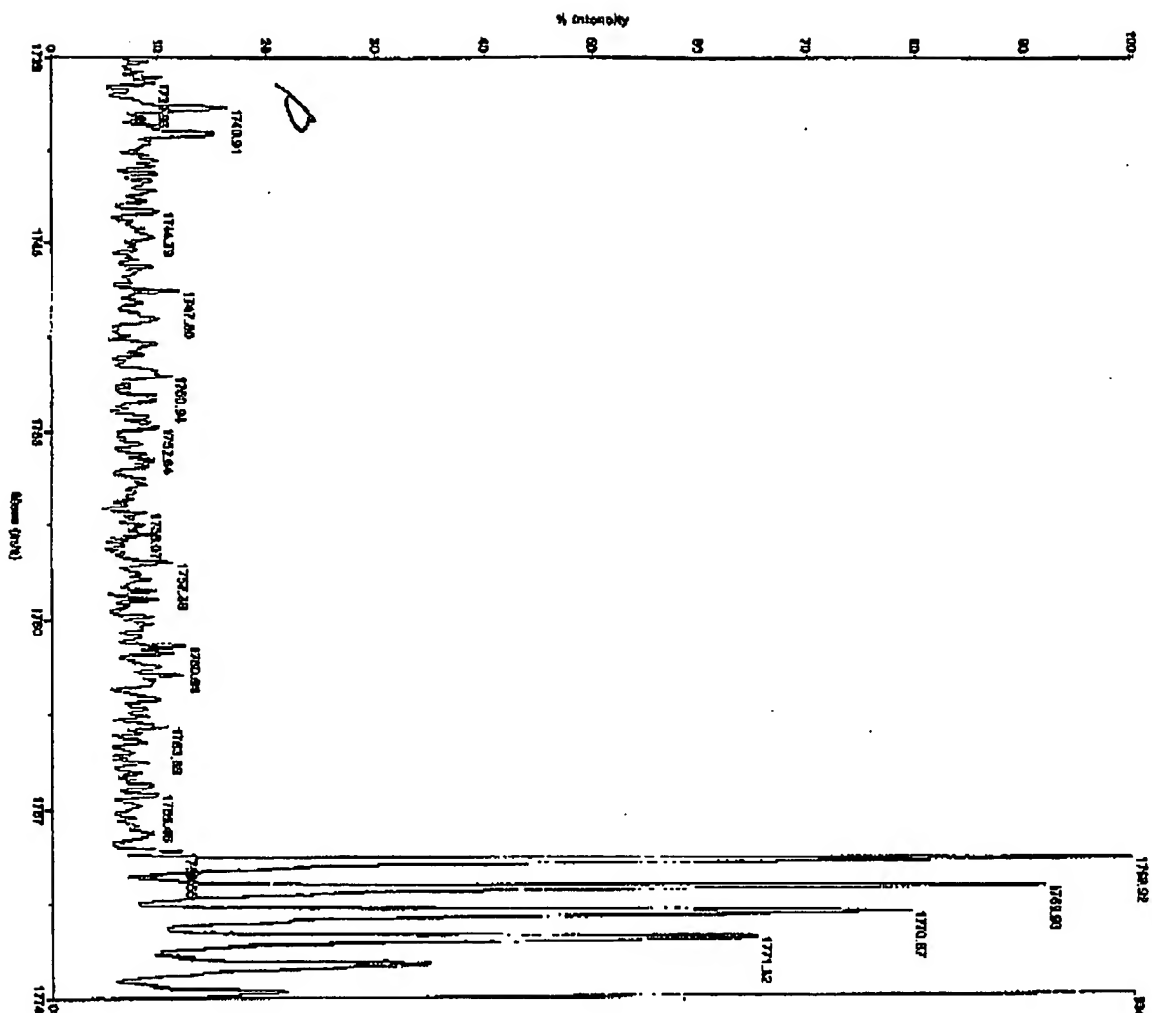
2

1

22

SP. sample high in β -myth protein
has high β protein as well
low α protein as well
 \therefore quantitate by NACD

Voyager Spec #1 MC88P = 17888, 89471



**Reflecting
Dehydrated
Potatoes
Manitoba!**

21000 V
78%
1.12
0%
100 msec

500 - 2500 Da
100/spectrum

5.8 Hz
External - 1H NMR prot. CDCl₃ DMSO-d₆ 11301_0001.cdf
3-Cyano-4-hydroxybenzoic acid
500 Da
O17

22.542
0.5 usoc
55420
1000 mV
0%
750 MHz

64
PLATE1
6137
Voyager-DE PRO
C:VOYAGER1100 well plate.ppt
Biochemical Mass Spectrometry Facility

17473.5
18691.3
646.011
-146.158
100
1.796e-007
4.614e-008
0.01192
B
679

GI sample kept in α -MgHC
has high & positive signal
lower positive signal
 \therefore generated by MACD